

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Miri Seiberg, et al.

Serial No. 10/659,598

Filed: December 7, 1998

Art Unit: 1614

Examiner: Shirley V. Gembeh

Attorney Docket No.: JBP 430CIP1

**METHOD FOR TREATING SKIN
CONDITIONS**

DECLARATION OF MIRI SEIBERG, PH.D.

I, Miri Seiberg, am a Distinguished Research Fellow in the Skin Research Center at Johnson & Johnson Consumer Companies, Inc. My education includes a Ph.D. in Molecular Biology from The Weizmann Institute of Science, Rehovot, Israel, in collaboration with Princeton University, Princeton, NJ and a B. S. in Life Sciences from Tel-Aviv University, Tel-Aviv, Israel. My curriculum vitae is attached hereto as Exhibit A.

1. "Lecithin" is the common name of phosphatidyl choline. However, in a broader usage the term lecithin describes the entire phospholipid fraction separated from soybean crude oil (Soybeans, chemistry, technology and utilization, by K. Lin, An Aspen publication, 1999, p. 32). (Attached hereto in Exhibit B)

2. The extraction and processing of soybean crude oil to obtain lecithin, as described e.g. by Lin (Soybeans, chemistry, technology and utilization, by K. Lin, An Aspen publication, 1999, chapter 6), starts with solvent extraction (p.297), and is most commonly performed with hexane extraction (p. 304-6). (Attached hereto in Exhibit B) Therefore, soy lecithins are generally known to be obtained by extraction with hexane.

3. Proteins are said to be "denatured" when their physical and physiological properties are changed such that they lose their activity. Such change is generally due to a change in a protein's chemical structure and/or conformation. Protein denaturation and the consequent loss of biological activity are not related to the source of the protein or to their origin, and are described in biochemistry textbooks (e.g. Biochemistry, A. L. Lehninger, 1975, p.62-63).

Matveev describes the dependence of denaturation time on organic solvent concentrations. Extraction with organic solvents was shown to denature many proteins (Sikorski and Naczki, 1981). In 1984, Benedek et al measured the kinetics of denaturation of several proteins, including soybean trypsin inhibitor (STI), as a function of the organic modifier employed. Khmel'nitsky et al (1991) documented the denaturation of several proteins by a broad series of organic solvents of different nature. van Erp et al (1991) developed a theoretical model, based on a generally accepted notion that the destruction of the protein hydration shell is one of the main reasons for protein denaturation by organic solvents. These studies document that proteins (including STI) are denatured in the presence of organic solvents. Copies of the foregoing references are attached hereto as Exhibit C.

5. Thus, I conclude that because soy lecithins are generally extracted utilizing hexane, an organic solvent, they would not be expected to contain non-denatured proteins such as STI as organic solvents are known to denature proteins.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Dr. Miri Seiberg



Date

Exhibit A

Miri Seiberg

168 Herrontown Rd. Princeton, NJ 08540
(908) 874-2325 (W); (609) 497-0148 (H)

Education

- 1977 B.Sc. Biological Sciences, Tel-Aviv University, Israel.
- 1982 M.Sc. Biochemistry, The Weizmann Institute of Science, Israel.
- 1989 Ph.D. Molecular Biology, The Weizmann Institute of Science, Israel, in collaboration with Princeton University, Princeton NJ.

Employment

- 1982 The Weizmann Institute of Science, Israel.**
Research assistant, Dept. of Chemical Immunology.

- 1982-90 Princeton University, Princeton NJ**
 - 1982-84 Visitor, Dept. of Biochemical Sciences.
 - 1987-89 Visitor, Dept. of Molecular Biology.
 - 1989-90 Post Doctoral Fellow, Dept. of Biology.

- 1990- 92 Bristol-Myers Squibb PRI, Princeton NJ.**
Post Doctoral Fellow, Dept. of Macromolecular Structure.

- 1992- Johnson & Johnson Family of Companies**
 - 1992-95 Senior Scientist, Skin Biology Research Center of Pharmaceutical Research Institute, Raritan NJ.
 - 1995-96 Staff Scientist, Dermatology R&D, Johnson & Johnson Consumer Companies, CPWW division, Skillman NJ.
 - 1997-99 Principal Scientist, Skin Research Center, CPWW, Skillman NJ
 - 1999-0 Research fellow, Skin Research Center, CPWW, Skillman NJ
 - 2001 -05 Sr. Research fellow, Skin Biology TRC and LAS, CPWW, Skillman NJ
 - 5/2005- Principal Research fellow, Skin Biology TRC and LAS, CPWW, Skillman NJ

Industrial Experience

1990- 92, Bristol-Myers Squibb PRI, Princeton NJ.

Post Doctoral Fellow, Dept. of Macromolecular Structure.

Using a rat model system for salt-induced hypertension, identified a novel gene involved in salt-induced hypertension, and demonstrated selective expression patterns.

1992-today, Johnson & Johnson Pharmaceutical Research Division

1992-95, Senior Scientist

This position involves conducting individual projects, supervising one BS/MS technician. Identified pathways involved in epidermal differentiation, hair growth and keratinocyte apoptosis. Developed relevant bioassays and screens.

Johnson & Johnson Consumer Companies, Inc.

1995-96, Staff Scientist

Directed two research scientists. Developed enzymatic, molecular and cellular assays and screens for potential drug and cosmetic activity. Involved in retinoid studies, proteases and protease inhibitors, in epidermal differentiation and hair growth.

1997-99, Principal Scientist

Head of pigmentation group. Directed research scientists and postdoctoral fellows. Horizontally directed the pigmentation technology development team. Initiated and directed molecular, cellular, and biochemical studies of pigmentation, resulting in the identification of a novel pathway that regulates skin color. Identified agents, both drugs and cosmetics, to modulate this pathway, resulting in darkening or lightening of human skin. Designed and evaluating product prototypes for biological activity and efficacy. In charge of numerous academic collaborations.

1999-00, Research Fellow

Continue heading the pigmentation team and supporting technology and product design groups in creating a line of depigmenting agents. First products available in stores. Additional responsibility in heading the hair growth efforts, introducing a new concept for delaying hair growth. Identified novel cosmetic agents with modulatory effect, demonstrated preclinical POP and initiated product development efforts. Expand responsibility for academic collaborations.

2001 -2005, Sr. Research Fellow

Director of the Skin Biology research group, including pigmentation, hair, acne, skin aging and skin cancer teams and supporting facilities. Continue basic research and product development support in all areas. Identified a novel cosmetic for skin aging, currently under early development stages. Directed efforts in the development of a new drug for acne, based on a proprietary target, now under clinical evaluation. Continue R&D support for skin lightening technology, now sold by numerous Brands and J&J companies worldwide. Continue R&D support for delaying hair growth technology, now sold by numerous J&J companies and Brands worldwide. Received the Johnson Medal, the highest level of scientific recognition by JJ. Head of Laboratory Animal Services, incl. vivarium support for numerous J&J companies. Council member of the J&J Corporate office of Science and Technology. In charge of academic interactions and collaborations for Skin Biology and related areas. In charge of the J&J SRC training grant. Member of the mentoring team.

5/2005 to present, Principal Research Fellow

M. Seiberg

Patent applications

More than 25 patent applications in the areas of skin and hair

J&J Awards

1. Skin care council – best scientific content poster award. June 1993.
2. American Express achievement award of PRI. January 1995.
3. COSAT-CORD internship award. April 1997.
4. Skin care council – best overall poster award. June 1999.
5. COSAT excellence in science award. November 1999.
6. CPWW achievement award. January 2000.
7. Skin care council – best overall poster award. June 2001.
8. CPPW Grandview award. March 2003.
9. The Johnson Medal. Oct 2003.
10. The Mountainview award. March 2005.

Societies

1. Pan American Society of Pigment Cell Research (council member, 2001-03, member of finance committee, 2000-02, nominated for 2005 presidency elections).
2. Society of Investigative Dermatology
3. American Society of Cellular Biology
4. American Association for the Advancement of science
5. New York Academy of Science (elected 2003)

SOYBEANS

Chemistry, Technology,
and Utilization

KeShun Liu

Soyfoods Laboratory
Hartz Seed, a Unit of Monsanto Company

A Chapman & Hall Food Science Book



An Aspen Publication®
Aspen Publishers, Inc.
Gaithersburg, Maryland
1999

The author has made every effort to ensure the accuracy of the information herein. However, appropriate information sources should be consulted, especially for new or unfamiliar procedures. It is the responsibility of every practitioner to evaluate the appropriateness of a particular opinion in the context of actual clinical situations and with due considerations to new developments. The author, editors, and the publisher cannot be held responsible for any typographical or other errors found in this book.

Aspen Publishers, Inc., is not affiliated with the American Society of Parenteral and Enteral Nutrition
Cover design: Rachael McBrearty

Library of Congress Cataloging-in-Publication Data

Liu, KeShun
Soybeans: chemistry, technology, and utilization / by KeShun Liu.
p. cm.
Originally published : New York : Chapman & Hall, 1997.
Includes bibliographical references and index.
(Formerly published by Chapman & Hall. ISBN 0-412-08121-0) ISBN 0-8342-1299-4
(alk. paper)

1. Soybean. 2. Soybean—Composition. 3. Soyfoods. 4. Soybean products

I. Title
SB205.S7L585 1997
664'.805655—dc21
96-44547
CIP

Copyright © 1997, 1999 by Aspen Publishers, Inc. All rights reserved.

Aspen Publishers, Inc., grants permission for photocopying for limited personal or internal use. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. For information, address Aspen Publishers, Inc., Permissions Department, 200 Orchard Ridge Drive, Suite 200, Gaithersburg, Maryland 20878.

C.H.I.P.S.

10777 Mazoch Road
Weimar, TX 78962 U.S.A.

Tel: 409 263-5683

Fax: 409 263-5685

www.chipsbooks.com

About Aspen Publishers • For more than 35 years, Aspen has a variety of disciplines. Aspen's vast information resources in many formats. We are committed to providing the highest quality information in the most appropriate format for our customers. Visit Aspen's Internet site for more information resources, directories, articles, and a searchable version of Aspen's full catalog, including the most recent publications: <http://www.aspenpublishers.com>

Aspen Publishers, Inc. • The hallmark of quality in publishing
Member of the worldwide Wolters Kluwer group

Editorial Services: Ruth Bloom
Library of Congress Catalog Card Number: 96-44547
ISBN: 0-8342-1299-4

Printed in the United States of America
2 3 4 5

composition, interesterification generally increases crystallization tendencies (melting point) of fats and oils. Details are provided in Chapter 6.

Reports differ regarding the effect of fatty acid distribution within triglyceride molecules on oxidative stability. Some reported that when specific fatty acids occupy the 1 and 3 positions of a triglyceride, oxidative stability is greater than when those same fatty acids occupy the 1 and 2 positions (Neff et al. 1992). Others found that the positional distribution has no effect on oxidative stability (Park et al. 1983).

B. Phospholipids

Crude soybean oil contains 1–3% phospholipids. Among the total phospholipids in soybeans, there are about 35% phosphatidyl choline, about 25% phosphatidyl ethanolamine, about 15% phosphatidyl inositol, 5–10% phosphatidic acid, and the rest is a composite of all the minor phospholipid compounds. Figure 2.4 shows the molecular structure and formation of three major types of phospholipids found in soybeans. The parent compound is phosphatidic acid, which is not present in the free form in active cells except as an intermediate in the biosynthesis of other phosphoglycerides. Others are esters of phosphatidic acid.

Both triglycerides and phospholipids are saponifiable but phospholipids are polar lipids. Removal of polar lipids from crude oil is carried out by centrifugation following hydration at an elevated temperature, the process commonly known as *degumming*. Phospholipids are good emulsifying agents, soluble in alcohol and insoluble in acetone. In living tissues, they are the major components of cell membranes.

It should be emphasized that phosphatidyl choline's common name is lecithin. However, in broad usage, the term "lecithin" generally refers to the entire phospholipid fraction separated from soybean crude oil by degumming. Lecithin processing and utilization are covered in Chapters 6 and 7, respectively.

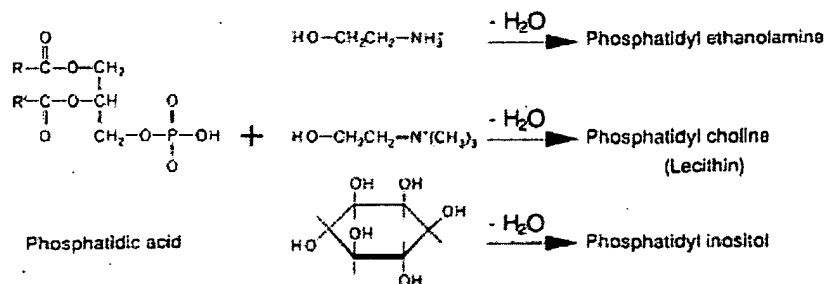


Figure 2.4. Molecular structure and formation of phospholipids commonly found in soybeans.

throughput, reduced solvent retention and nonhydratable phosphatides in the oil. Unfortunately, this is at the cost of lower oil extractability.

II. Solvent Extraction

During solvent extraction oil is removed from the soy flakes by an organic solvent to form an oil/solvent mixture called a *miscella*. The oil is recovered from the miscella by removing the solvent by steam stripping.

A. Solvents

Most commercial oil extraction is currently by hexane extraction, but other solvents have been considered. Snyder and Kwon (1987) reviewed the desirable properties of the oil solvent, which were also discussed by Lusas et al. (1989). Ideal solvent properties would be triglyceride selectivity, nontoxic residues in the oil, a low specific heat, low heat of vaporization, and unreactivity with oilseeds and extraction equipment. The ideal solvent would not be flammable or explosive and would be cheap and available in large quantities.

Such an ideal solvent does not exist but hexane fulfills most of the requirements, therefore, it is widely used for soy oil extraction. Some properties and specifications of hexane used for soy oil extraction are shown in Table 6.1. A recent risk assessment of hydrocarbon solvents in oil processing claimed hexane to be as safe as other hydrocarbons (Galvin et al. 1995). Unfortunately, hexane is explosive, flammable, and expensive, so there have been studies over the years to investigate alternative solvents. In the 1940s large-scale soy oil solvent extraction was in its infancy and concern regarding the use of hexane led to the investigation of trichlorethylene, which was used as an extraction solvent in the early 1950s. However, its use was quickly discontinued after toxic effects of the solvent were shown after feeding the extracted soy meal to cattle. The toxin was a derivative of cysteine called *s-trans*-dichlor-vinyl-L-cysteine. Death occurred when the animals were fed 2–3 lbs of soy meal per day (Snyder and Kwon 1987). This has caused chlorohydrocarbons to be largely avoided as extraction solvents.

Alcohols have been and are being examined as alternatives to hexane, although at room temperature they dissolve very little triglyceride. Oil solubility can be greatly improved with an increase in temperature or the addition of water (Fig. 6.6). Therefore, oil extracted at high temperatures can be separated from the solvent by cooling. There may be some residual oil in the solvent that can be stripped out at a cost less than that of removing solvent from the whole crude oil (Johnson and Lusas 1983).

A four-stage alcohol extraction process has been described (Karnofsky 1981) whereby flakes and solvent are moved countercurrently. Full fat flakes are first extracted with dilute alcohol, which removes fatty acids, phosphatides and carbohydrates, but not the oil. The flakes are then incubated with 100% alcohol and

fin
Fin
des
/
Sul
and
oil
ext
lact
aze
pro
stuc
inst
C
USI
alth

Table 6.1 Example of Purchase Specifications for Hexane To Be Used for Extraction of Soybean Oil

Property	Value
Specific gravity at 25°C (g/cm ³)	0.6705–0.6805
Distillation range (760 mm)	
Minimum initial boiling point (°C)	65.0
Typical 10% distillation (°C)	67.1
Typical 50% distillation (°C)	67.7
Typical 90% distillation (°C)	68.2
Maximum dry point (°C)	70.0
Maximum nonvolatile residue (g/100ml)	0.001
Acidity of distillation residue	Neutral
Closed-cup flash point (°C)	–32 to –58
Maximum sulfur	10
Maximum vapor pressure (psia at 35°C)	6.0
Composition (GLC % area) ^a	
<i>n</i> -Hexane	45–70
Methyl cyclopentane	10–25
Total <i>n</i> -hexane and methyl cyclopentane	60–80
Total 2-methyl pentane; 2,3 dimethyl butane; and 3-methyl pentane	18–36
Maximum cyclohexane	2.5
Maximum benzene	0.1
Maximum APHA color	15
General appearance	Free of foreign matter

Source: From Johnson and Lusas (1983).

^aGLC = gas liquid chromatography.

^bAPHA = American Public Health Association

finally the oil is removed by a constant boiling azeotrope of water and alcohol. Finally, residual oil in the flakes is extracted with distilled alcohol obtained from desolventized flakes and oil stripping.

Aqueous isopropanol has been studied as an extracting solvent (Baker and Sullivan 1983). An azeotrope of 87.7% isopropanol was effective at 77°C (170°F) and the oil phase was separated by cooling to 30°C (86°F), with a reduction of oil phospholipid and free fatty acid content, relative to oil obtained by hexane extraction. However, the effectiveness of the process has been limited by the lack of means to recover isopropanol in greater concentrations than the water azeotrope (87.8%) (Lusas et al. 1994). An integrated distillation pervaporation process has been proposed to improve the recovery (Lusas et al. 1994). Later studies investigated the use of membranes to improve isopropanol recovery instead of plate and frame membranes (Lusas et al. 1995).

Carbon dioxide has been studied as a potentially safe, nontoxic solvent at the USDA Northern Regional Center in Peoria, Illinois. Pressurized carbon dioxide, although not liquified, acquires some liquid flow and solvent properties, and is

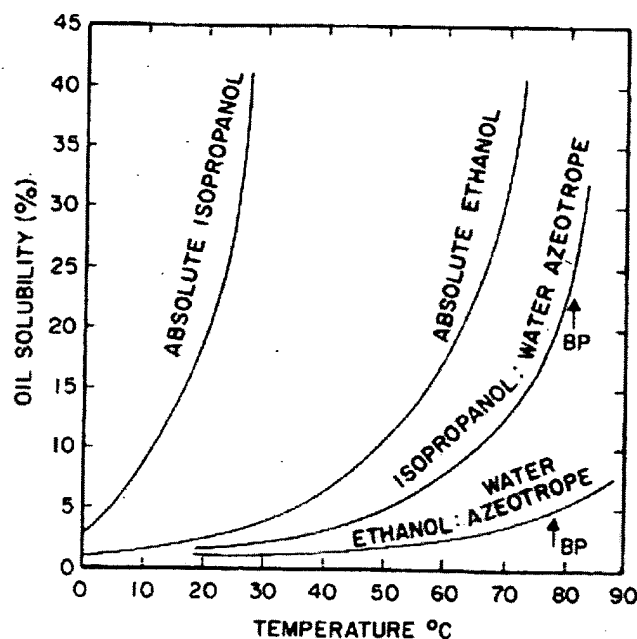


Figure 6.6. Solubilities of oil in different solvent systems. From Johnson and Lusas (1983).

named supercritical fluid (Freidrich and Pryde 1984). Carbon dioxide extracted oil contains less phospholipids than hexane extracted oil, but they are otherwise similar. Moisture content and particle size correlated with extraction rate and oil yield at 50°C and 8000 psia (Snyder et al. 1984). The increased expense of carbon dioxide extraction is due to the expensive equipment and the need to generate high pressure (1000–10,000 psia). However, efforts have been made to reduce the costs (Reverchon and Osseo 1994).

B. Extraction Theory

Solvent extraction of soybeans is a diffusion process in which the solvent (hexane) selectively dissolves miscible oil components. During extraction, hexane solubilizes soy oil from cotyledon lipid bodies in soy flakes rapidly, as soon as it enters the lipid body. The slowest process is solvent diffusion into the flake and diffusion of the oil/hexane miscella out. Nevertheless, this process is faster than extraction of raw cotyledons or fresh beans, which are almost impenetrable by hexane.

Flake thickness is therefore very important in controlling diffusion. Flakes must be thick enough to avoid breaking up during handling. Crumbling of the thin flakes will result in fines that will not allow the solvent to flow through the

fine part
(0.010–0
extractio

The oil c
constant.
-dC/dt (
Norris
during t
This diff
initially.
of the fl
does not

The m
coefficient
increase,
controlli
minor co
continue
Karnofsl
concentr
scope of
time is l
(1950) r
greater t

C. Extra

Several
by conti

The R
rent syst
segment
through
process
more fla
before a
flakes a
system.
of each
flake de

The L
rent ext

Exhibit C

Tsitologiia. 1975 Nov;17(11):1278-82.

Denaturation time of actomyosin exposed to different chemicals

Matveev VV

The frog skeleton muscle actomyosin denaturation time dependence on the concentration of salts (NaCl and CaCl₂) and organic chemicals (carbohydrates, narcotics and alcohols) was investigated. The following effects were detected: phase change in denaturation time associated with the rise of concentration of chemicals under study; actomyosin stabilization effect; coincidence of concentrations giving rise to protein stability with those increasing the survival time of isolated frog skeleton muscles in vitro (literature data); denaturation effect of alcohols used, both in high and very low concentrations.

Crit Rev Food Sci Nutr. 1981;14(3):201-30.

Modification of technological properties of fish protein concentrates.

- Sikorski ZE
- Naczek M

Fish protein concentrates are mixtures of cross-linked and aggregated molecules of different muscle proteins. The final conformation of the components of the mixtures is formed as a result of procedures applied to convert the raw materials into a product of desirable and stable sensory properties, containing less than 0.1% of lipids. To achieve this end usually extraction with hot organic solvents, mainly isopropyl alcohol and 1,2-dichloroethene, followed by air drying are employed. These conditions bring about denaturation of many of the proteins followed by aggregation of the molecules due to the interaction of reactive functional groups in extended polypeptide chains. In the final product a large proportion of hydrophobic groups is exposed to the solvent and the proteins exhibit an extremely low water affinity. Such concentrates, although valuable as protein supplements, have only limited suitability as active components of various processed foods, as they have poor technological value. They are insoluble or have a very low water dispersibility and swelling ability, do not form gels after heating, or have any significant fat-emulsifying capacity. Changing the dissociation or number of ionic groups of the molecules prior to extraction, e.g., by acidifying or acylating, can partially reduce the denaturing effect of heat and organic solvents and thus improve the functional properties of the product. An upgrading of the quality of concentrates produced by hot extraction can be achieved by partial enzymatic or chemical deaggregation, hydrolysis followed by the plastein reaction, or formation of suitable derivatives. The best results have been obtained by partial hydrolysis of acylated proteins or precipitation of the aggregated products using sodium hexametaphosphate. The functional properties of such products are comparable to those of vegetable protein isolates used as meat extenders. Various proteins of high technological value can be also obtained by enzymatic hydrolysis of the raw material, followed by separation of the lipids without organic solvent extraction. Such products, however, have a distinct odor and flavor and must be stabilized because of residual lipids.

J Chromatogr. 1984 Dec 28;317:227-43.

Kinetics of unfolding of proteins on hydrophobic surfaces in reversed-phase liquid chromatography.

- Benedek K
- Dong S
- Karger BL

As a continuation of previous studies, we present in this paper measurements on the kinetics of denaturation of papain, soybean trypsin inhibitor and lysozyme on n-butyl-bonded silica gel surfaces used in reversed-phase liquid chromatography (RPLC). In all cases, native and denatured peaks widely separated from one another are observed. The rate constants for denaturation or unfolding are determined by the measurement of the peak area of the native protein as a function of the incubation time that the species spends on the bonded-phase surface. The results reveal that a slow denaturation step occurs with a half-life of ca. 15 min. In addition, studies of denaturation as a function of the amount of 1-propanol in the initial mobile phase suggest an additional unfolding step when the protein comes in contact with the bonded-phase surface. The extent of this latter step decreases as the concentration of 1-propanol increases, further suggesting that 1-propanol sorption on the bonded stationary phase may play a role in this behavior. Other studies are conducted with alpha-chymotrypsinogen, in which injection is made after the start of the gradient. The extent of denaturation is observed to be a function of the organic modifier employed. The results of this paper provide insight into the denaturation process in RPLC and suggest approaches to minimize this behavior.

Eur J Biochem. 1991 May 23;198(1):31-41.

Denaturation capacity: a new quantitative criterion for selection of organic solvents as reaction media in biocatalysis.

- Khmelnitsky YL
- Mozhaev VV
- Belova AB
- Sergeeva MV
- Martinek K

A. N. Bakh Institute of Biochemistry, Moscow, USSR.

The process of reversible denaturation of several proteins (alpha-chymotrypsin, trypsin, laccase, chymotrypsinogen, cytochrome c and myoglobin) by a broad series of organic solvents of different nature was investigated using both our own and literature data, based on the results of kinetic and spectroscopic measurements. In all systems studied, the denaturation proceeded in a threshold manner, i.e. an abrupt change in catalytic and/or spectroscopic properties of dissolved proteins was observed after a certain threshold concentration of the organic solvent had been reached. To account for the observed features of the denaturation process, a thermodynamic model of the reversible protein denaturation by organic solvents was developed, based on the widely accepted notion that

an undisturbed water shell around the protein globule is a prerequisite for the retention of the native state of the protein. The quantitative treatment led to the equation relating the threshold concentration of the organic solvent with its physicochemical characteristics, such as hydrophobicity, solvating ability and molecular geometry. This equation described well the experimental data for all proteins tested. Based on the thermodynamic model of protein denaturation, a novel quantitative parameter characterizing the denaturing strength of organic solvents, called the denaturation capacity (DC), was suggested. Different organic solvents, arranged according to their DC values, form the DC scale of organic solvents which permits theoretical prediction of the threshold concentration of any organic solvent for a given protein. The validity of the DC scale for this kind of prediction was verified for all proteins tested and a large number of organic solvents.

European Journal of Biochemistry, Vol 202, 379-384, Copyright © 1991 by Federation of European Biochemical Societies

The effect of water content and nature of organic solvent on enzyme activity in low-water media. A quantitative description

SH van Erp, EO Kamenskaya and YL Khmelnsky

A. N. Bakh Institute of Biochemistry, Moscow, USSR.

A simple theoretical model was suggested to describe quantitatively the effect of water content and nature of organic solvents on catalytic behavior of enzymes suspended in low-water media. The model was based on a generally accepted notion that the destruction of the protein hydration shell is one of the main reasons for protein denaturation by organic solvents. The validity of the model was confirmed by the example of catalytic behavior of immobilized laccase suspended in water/organic mixtures of different compositions. In addition, the results were used to demonstrate that the effect of organic solvents and/or water content on catalytic behavior of enzymes in low-water media can be adequately assessed only in terms of the full kinetic description based on properly determined V_m and K_m values.

Process for the isolation and purification of isoflavones

United States Patent 5679806 (Filed 02/24/1995, granted 10/21/1997)

Abstract: The present invention relates to a process for the isolation and purification of isoflavones from a number of different biomass sources. More particularly, the present invention relates to a three-step process whereby a biomass containing isoflavones with a solvent thereby forming an extract that is subsequently fractionated using a reverse phase matrix in combination with a step gradient elution, wherein the resulting fractions eluted from the column contain specific isoflavones that are later crystallized. The purified isoflavone glycosides may then be hydrolyzed to their respective aglycone.